Effect of T Cell Modulation on the Translocation of Bacteria from the Gut and Mesenteric Lymph Node

MICHAEL A. MADDAUS, M.D.,* CAROL L. WELLS, PH.D.,*† JEFFREY L. PLATT, M.D.,‡
RICHARD M. CONDIE, M.S.,* and RICHARD L. SIMMONS, M.D.*

Although the ability of the gut-associated lymphoid tissue (GALT) to respond to orally ingested foreign antigens has been studied extensively, its function in preventing or limiting escape of resident gut bacteria has not been assessed. The following studies were performed to examine what role cell-mediated immunity (CMI) plays in this process. The ability of suppression of CMI to induce escape of gut bacteria (translocation) to the mesenteric lymph node (MLN) in immunocompetent mice whose gut flora was unaltered was examined. Administration of cyclosporine or anti-L3T4 antibody failed to induce translocation of indigenous gut bacteria after 7 or 14 days of treatment. Antithymocyte globulin (ATG) also failed to induce translocation after 7 days of treatment, despite depletion of all Thy 1, Lyt 1, L3T4, and Lyt 2 positive cells from the spleen, MLN, and intestine as demonstrated by immunofluorescent microscopy. Finally, cultures of the MLN, spleen, liver, and peritoneum of T cell-deficient BALB/c nude mice and their heterozygous T cell-replete littermates were also sterile, demonstrating that congenital suppression of T CMI also does not lead to translocation of indigenous gut bacteria. The role of CMI in limiting systemic spread of bacteria that were already translocating to the MLN was also examined. Translocation of *Escherichia coli* C25 to the MLN was induced by gastrointestinal (GI) monoassociation, which leads to translocation of *E. coli* C25 to the MLN in 80–100% of mice. Treatment with ATG during monoassociation failed to induce spread of *E. coli* C25 to the spleen, liver, or peritoneum, despite the same degree of T cell depletion achieved with ATG in the previous experiment. Monoassociation of conventional T cell-deficient BALB/c nude and heterozygous mice and germ-free T cell-deficient BALB/c nude and heterozygous mice also did not lead to spread of *E. coli* C25 beyond the MLN. However, in ATG-treated, conventional nude, and germ-free nude mice, the average number of translocating *E. coli* C25 per MLN was consistently higher. In separate experiments the ability of stimulation of T cell function to inhibit translocation of *E. coli* C25 was examined. Recombinant interleukin-2, 25,000 units, was administered intraperitoneally every 8 hours during exposure to *E. coli* C25. This reduced the incidence of translocation of *E. coli* C25 from 85% to 51% ($p = 0.02$). Suppression of CMI, either systemically or within the GALT, has a minimal influence on the mechanisms by which the normal gut flora are translocated to the MLN. However, suppression of CMI promotes increased survival of the bacteria that have translocated to the MLN. Conversely, augmentation of T cell function may reduce the number of bacteria that survive in the node, possibly by enhanced bacterial killing within the local environment of the MLN.

From the Departments of Surgery, Laboratory Medicine and Pathology,*† and Pediatric Nephrology,‡ University of Minnesota, Minneapolis, Minnesota*

HOSPITAL-ACQUIRED or nosocomial infection continues to be one of the leading causes of death in critically ill surgical patients. Whether following trauma, burns, or routine intra-abdominal surgery, the majority of these infections are due to gram-negative bacilli, enterococci, *Candida albicans,* and coagulase-negative staphylococci. Coagulase-negative staphylococcal infections are most often due to colonization of intravascular monitoring devices. Infections caused by gram-negative bacilli, enterococci, and *C. albicans,* microbes that are normally found primarily in the gastrointestinal (GI) tract, appear at a variety of sites including the lung, peritoneal cavity, sinuses, and urinary tract. Recently, however, it has been recognized that the GI tract, due to an acquired inability to confine microbes, may also serve as a reservoir for systemic infection in these patients.[1] Because surgical patients frequently suffer from malnutrition, gram-negative infection, shock (with compromised mesenteric blood flow), ileus with its attendant stasis of intestinal contents, and lack of oral intake, the capability of the gut to maintain

Supported in part by NIH Grant Nos. AI 14032 and AI 23484. Dr. Platt is under tenure of a Clinician–Scientist Award from the American Health Association.

Reprint requests and correspondence: Michael A. Maddaus, M.D., Department of Surgery, Box 230 Mayo Building, University of Minnesota, Minneapolis, MN 55455.

Submitted for publication: September 17, 1987.

its mucosal and immune barriers against the escape of microbes (referred to as translocation), may be severely compromised. Since many of these predisposing factors are often found together, systemic escape of gut microbes or toxins may have important consequences in such critically ill patients.

Although all of these factors may lead to changes in gut structure and function, it is unknown what impact depression of immune function has on the GI tract's ability to prevent escape of bacteria. In particular, many abnormalities of T cell function occur following burns, trauma, and sepsis including decreased total T cell number, diminished responsiveness to the T cell mitogen PHA and to the lymphokine interleukin-2 (IL-2), and the generation of suppressor T cells. In burn patients the degree of depression of the T cell response to PHA has been shown to correlate with both the degree of burn injury and with the risk of developing infection and of dying from infection.[2] In preoperative patients, the status of their T cell function, as measured by skin testing to ubiquitous antigens, is highly predictive of the risk of developing a life-threatening postoperative infection and of the risk of death from sepsis.[3]

Because T cell function is intimately involved with many aspects of the immune response, and because depression of T cell function is strongly correlated with an increased risk of nosocomial infection after surgery or trauma, we became interested in the role of the T cell in the control of bacterial translocation from the GI tract. We performed the following studies to determine what role, if any, T cell immunity plays both in the normal process of confining bacteria to the gut and in the prevention of systemic dissemination of bacteria that have translocated to the lymph nodes that drain the GI tract.

Materials and Methods

Animals

Female Swiss Webster mice, 18–22 g (Bio-Lab Corporation, St. Paul, MN) were used in experiments involving administration of cyclosporine (CSA), anti-L3T4 antibody, and antithymocyte globulin (ATG) alone. Female BALB/c mice, 18 to 22 grams, (Department of Laboratory Medicine and Pathology mouse colony, University of Minnesota, Minneapolis, MN) were used in the experiments combining ATG and IL-2 administration with *E. coli* C25 monoassociation. Congenitally athymic nude (nu/nu) and heterozygous (nu/+) BALB/c mice, 8–11 weeks of age, were obtained from Life Sciences, Jacksonville, FL. Germ-free, 18–22 g, female BALB/c nude and heterozygous mice were housed and treated at the University of Wisconsin Gnotobiotic Laboratory, Madison, WI.

Bacteriologic Methods

After death, a swab culture of the peritoneal surface was taken and then the mesenteric lymph nodes (MLN), and, when indicated, the spleen and liver, were excised aseptically. The cecum was then removed, weighed, and transferred immediately to an anaerobic chamber (Forma Scientific, Marietta, OH). The MLN, spleen, and liver were homogenized with autoclaved glass stoppers in brain-heart infusion broth (Difco Laboratories, Detroit, MI) (MLN in 2 mL; spleen and liver in 4 mL). Two hundred microliters of the homogenate was plated in duplicate on 5% sheep blood agar (DiMed Inc, St. Paul, MN) and incubated at 35 C for 48 hours, after which the number of colony forming units (CFU) present per organ was determined. The peritoneal swab was incubated at 35° in 4 mL of brain-heart infusion broth for 24 hours following which 200 μL was plated in duplicate on 5% sheep blood agar and incubated at 35 C for 24 hours. Ceca were homogenized in 9 mL of prereduced Hank's balanced salt solution (Gibco Laboratories, Grand Island, NY). After serial dilution, 20 μL of each dilution was plated on each of the following media: (1) eosin-methylene blue agar (Difco) for enumeration of facultative gram-negative bacilli; (2) colistin-nalidixic acid agar (Difco) for enumeration of aerobic and facultative gram-positive bacteria; (3) Wilkins-Chalgren agar (Difco) supplemented with 0.1 mg/mL of gentamicin for enumeration of strictly anaerobic bacteria, and, where appropriate; and (4) nutrient agar (Difco) supplemented with 0.1 mg/mL of streptomycin sulfate (Sigma Chemical Co., St. Louis, MO) for enumeration of *E. coli* C25. After incubation for 24–48 hours, plates were read and total CFU/g of cecal weight was determined.

Gut Monoassociation with E. Coli *C25*

Mice were intestinally decontaminated by adding 2 mg/mL of streptomycin (Sigma) and 2 mg/mL of bacitracin (Sigma) to their drinking water for 4 days. An overnight culture of streptomycin-resistant *E. coli* C25 (provided by Dr. Rodney Berg, LSU Medical Center, Shreveport, LA) in brain-heart infusion broth (10^9 CFU/mL) was then added to the antibiotic-containing drinking water in a 1:1 dilution. Mice were killed after 48 hours of exposure to *E. coli* C25. In separate experiments, germ-free nude and heterozygous mice were orally inoculated with *E. coli* C25 without prior antibiotic treatment and 48 hours later were transferred by overnight air freight to our laboratory in shipping containers designed to maintain gonotobiotic conditions. The peritoneum, MLN, spleen, and liver were then cultured as described above.

Immunosuppressive Agents

Cyclosporine (CSA; Sandoz, Basel, Switzerland, 100 mg/mL) was diluted in olive oil to the desired concentration and administered subcutaneously in the flank once a day. CSA whole blood levels were determined by high pressure liquid chromatography by the Clinical Chemistry Laboratory, University of Minnesota Hospitals, Minneapolis, MN.

Anti-L3T4 antibody was derived from the GK1.5 monoclonal cell line (ATCC-TIB 507) from which antibody-rich supernatant was harvested and stored at −70 C. Aliquots were thawed just prior to use, and 0.5 mL of undiluted antibody supernatant or media control was injected daily into the dorsal tail vein. To determine the per cent of L3T4-positive cells remaining after 14 days of treatment, pooled cervical, axillary, inguinal, and MLN single-cell suspensions from three control and three L3T4-treated mice were incubated with anti-L3T4 antibody (1:2 dilution of monoclonal cell culture supernatant) and then counterlabeled with a 1:50 dilution of fluorescein-labeled antimouse immunoglobulin antibody (Cappel Laboratories, West Chester, PA) and then subjected to fluorescent activated cell sorter analysis to determine the percentage of L3T4-positive cells. The

TABLE 1. *Effect of CSA, Anti-L3T4 Antibody, and ATG on the Translocation of Indigenous GI Bacteria to the MLNs*

Treatment	Dose	No. of Mice with Translocation/Total No. of Mice* Day 7	Day 14
CSA	0	0/20	0/20
	10 mg/kg/d	1/20	0/20
	50 mg/kg/d	2/20	2/20
Anti-L3T4	0	1/10	1/10
	0.5 mL/d	1/10	1/9
ATG	0	2/20	ND
	1 mg/d	1/10	ND
	3 mg/d	3/10	ND
	6 mg/d	2/10	ND

* $p > 0.05$ for CSA, Anti L3T4, and ATG treatments compared to control by chi square analysis with a Yates continuity correction.
ND = not done.

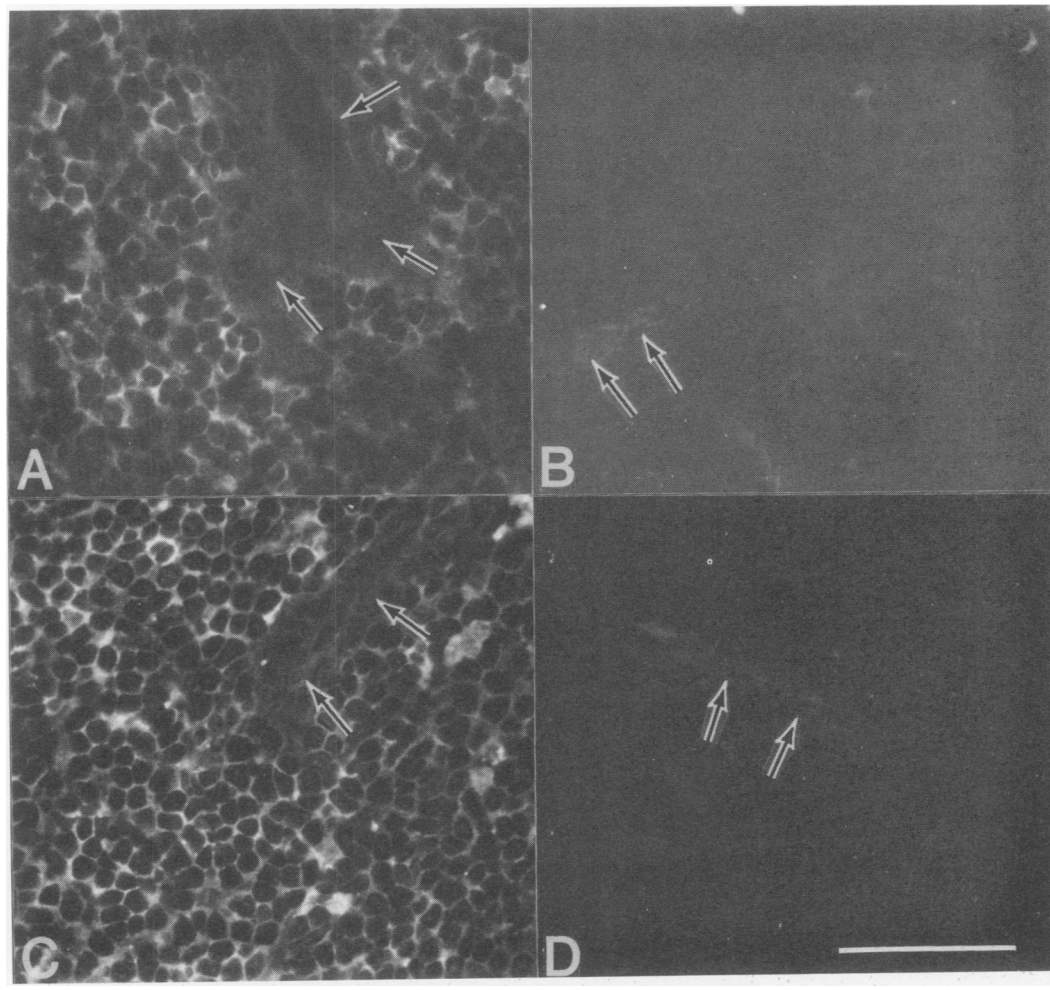

FIG. 1A–D. Frozen sections of spleen after staining with anti-Thy 1 (*A* and *B*) and with anti-Lyt 2 (*C* and *D*) (control: *A* and *C*; ATG-treated: *B* and *D*) demonstrating complete depletion of these cells. Staining with anti-L3T4 and anti-Lyt 2 antibodies also showed a complete absence of these T cell phenotypes. Scale bar represents 50 μm.

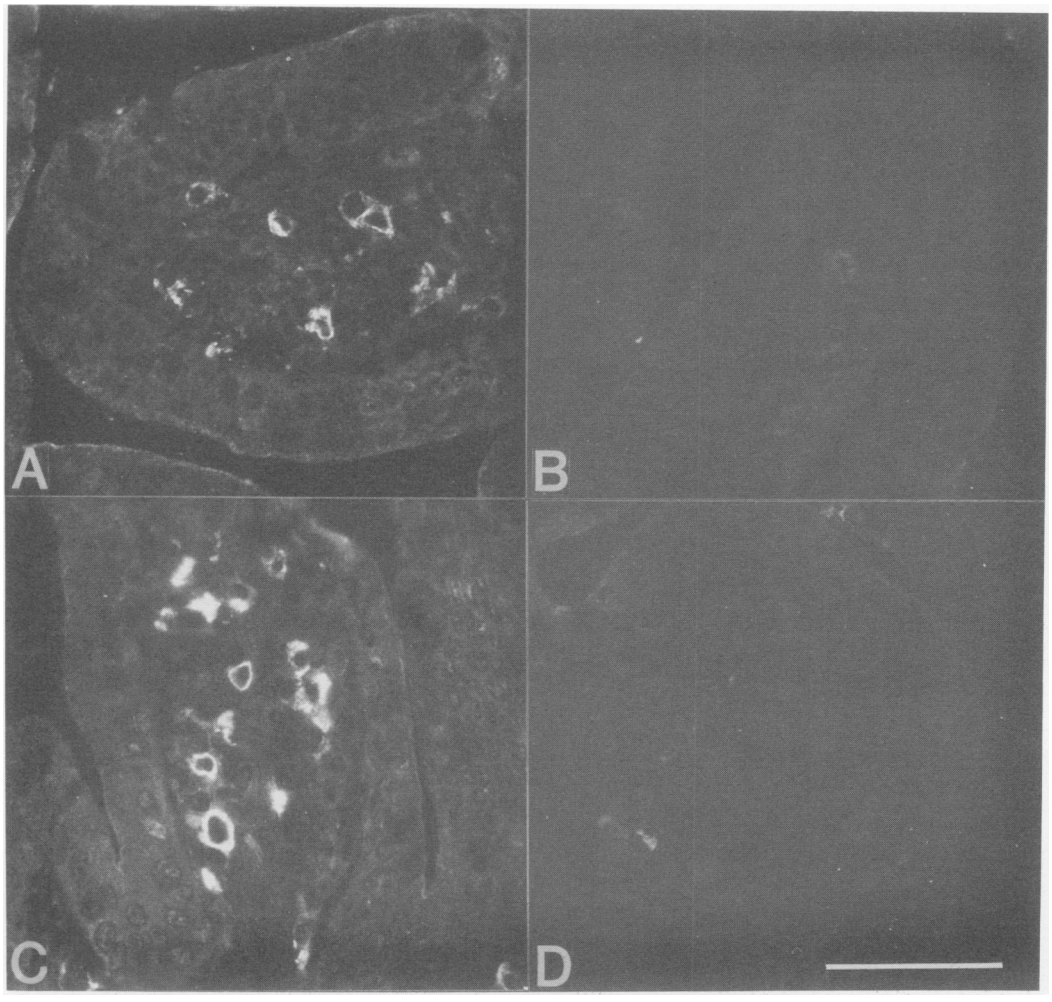

FIG. 2A–H. Frozen sections of small intestine after staining with anti-Thy 1 (*A* and *B*), anti-Lyt 1 (*C* and *D*), anti-L3T4 (*E* and *F*), and anti-Lyt 2 (*G* and *H*) (control: *A, C, E,* and *G;* ATG treated: *B, D, F,* and *H,* demonstrating complete depletion of these T cell phenotypes from the small intestine). Scale bar represents 50 μm.

average number of L3T4-positive cells remaining was expressed as a per cent of those found in control mice.

ATG (provided by Richard Condie, Director, Minnesota ALG Program, ALG Laboratories, Minneapolis, MN) is a pooled polyclonal IgG preparation derived from the immunization of horses with mouse thymocytes. The antibody was diluted in normal saline to the desired concentration, and 0.5 mL was administered intravenously (I.V.) daily via the dorsal tail vein.

IL-2 is a purified recombinant human lymphokine obtained from Cetus Corporation, Emoryville, CA. Appropriate dilutions were made in Hank's balanced salt solution without calcium or magnesium and administered intraperitoneally in a volume of 0.25 mL.

Immunofluorescent Antibody Studies

Tissues were snap frozen in precooled isopentane and stored at −70 C until used. Frozen tissue sections (4-μm thickness) prepared in a Lipshaw cryostat were stained using an indirect immunofluorescence technique previously described in detail.[4] Briefly, sections were fixed with acetone, washed in phosphate-buffered saline, and then reacted with an appropriately diluted monoclonal antibody and then reacted sequentially with the following: (1) affinity isolated fluoresceine isothiocyanate (FITC) F(ab′)2 conjugated goat-antirat IgG (H and L); (2) affinity isolated (FITC) F(ab′)2 rabbit antigoat IgG (H and L); and (3) p-phenylenediamine to retard fluorescence fading. FITC conjugated antibodies were absorbed with mouse serum prior to use. For each tissue, control sections were prepared as described above, omitting application of the monoclonal antibody.

Rat monoclonal antibodies were obtained from the following sources: anti-Thy 1 was obtained from Boehringer, Indianapolis, IN; anti-Lyt 1 (CD5) (53-7.3) and anti-Lyt 2 (CD8) (53-6.7) were from Becton Dickinson, Mountainview, CA; and anti-L3T4 (CD4) (GK1.5) was from ATCC. Antirat antibodies were from Pel-Freeze, Rogers, AR, and antigoat antibodies were from Cooper, Malvern, PA.

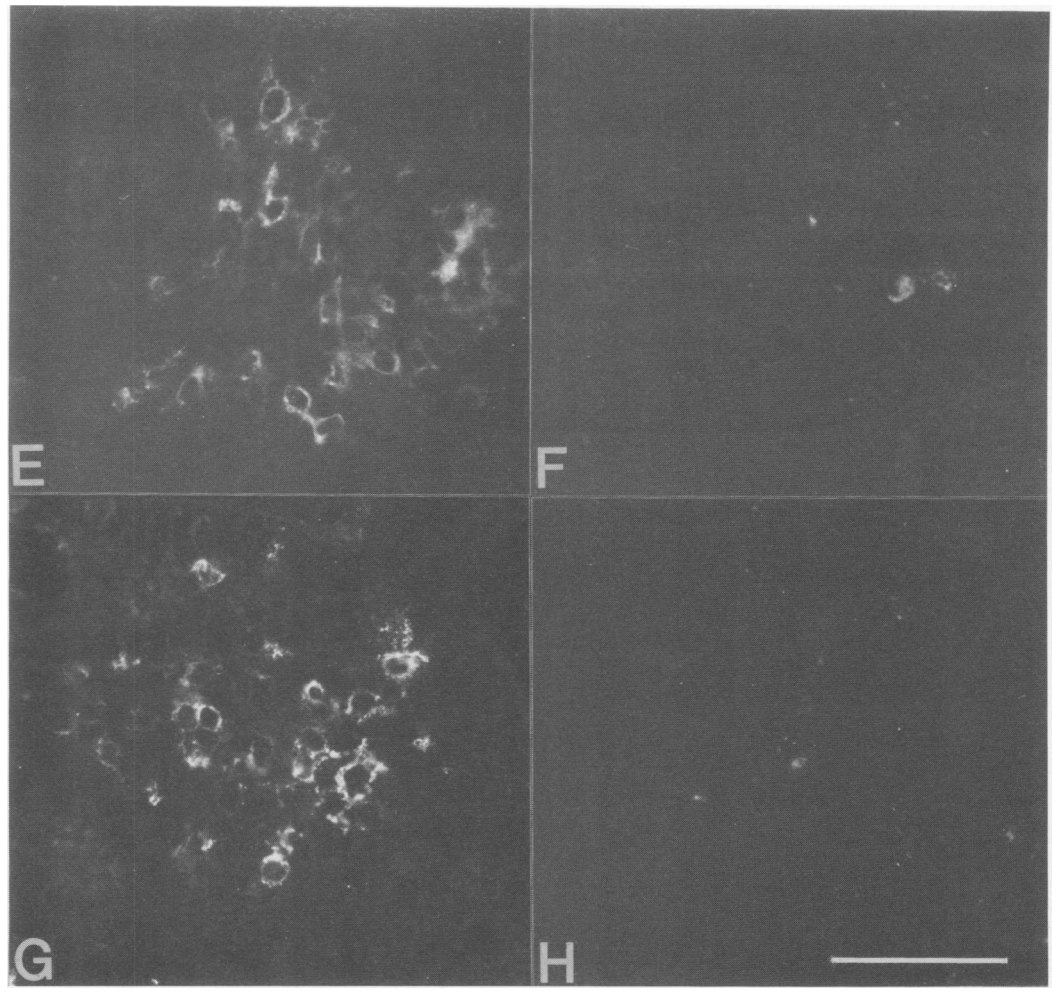

FIG. 2. (*Continued*)

Results

Effect of Suppression of Cell-mediated Immunity on Translocation in Immunocompetent Mice with a Normal Gut Flora

The first series of experiments was done to evaluate the ability of several T cell specific immunosuppressive agents to induce translocation from the gut to the MLN of Swiss Webster mice with a stable GI flora. In our laboratory, these mice exhibit a spontaneous translocation rate to the MLN of 0–10%. We first attempted to specifically suppress T-helper cell function through the use of CSA and anti-L3T4 antibody. CSA, which inhibits T-helper cell production of IL-2 and gamma interferon,[5] was administered daily in doses of either 10 mg/kg (a dose approximating that used for clinical organ transplantation) or 50 mg/kg (a more toxic dose). Control animals received olive oil devoid of CSA. On day 14 the average CSA whole blood level (taken 24 hours after the last dose) as determined by high pressure liquid chromatography was 160 ± 19 and 704 ± 67 mcg/liter (N = 3), respectively. Cultures of the MLN on days 7 and 14 demonstrated that translocation was not induced by CSA, despite moderate to high whole blood CSA levels (Table 1).

L3T4 is a cell surface molecule on mouse T-helper cells, which, through its interaction with class II major histocompatability antigens, facilitates recognition of foreign antigens.[6] Anti-L3T4 monoclonal antibody, when given *in vivo,* depletes the blood, spleen, and lymph nodes of L3T4 positive cells by 75–90% at 48 hours and 1 mg/wk can sustain a <90% reduction indefinitely.[7] In this experiment, 0.5 mL of undiluted hybridoma supernatant was administered I.V. daily for 14 days. Fluorescent activated cell sorter analysis of peripheral lymph node cells on day 14 demonstrated an average reduction of L3T4-positive cells of 75%. Cultures of the MLN on days 7 and 14 demonstrated that depletion of T-helper cells also failed to induce translocation (Table 1).

TABLE 2. *Incidence of Translocation of Indigenous GI Bacteria to the MLNs, Spleen, Liver, and Peritoneal Cavity of Athymic (Nude) Mice and Heterozygous (Thymic) Littermates*

Type of Mouse	Site of Bacterial Translocation			
	MLN	Spleen	Liver	Peritoneal Cavity
Heterozygote	2/20	0/20	0/20	0/17
Nude	0/18	1/18	0/17	1/16

Values are given as no. of mice with translocation of indigenous bacteria/total no. of mice.

Since neither T-helper cell inhibition (CSA) nor depletion (anti-L3T4 antibody) induced translocation, we next sought to more broadly deplete the animals of T cells with ATG. In our laboratory, when used as the sole immunosuppressive agent in mice, four 0.5 mg doses of ATG prevent rejection of skin grafts across major histocompatability barriers for an average of 25 days. One, three, and six mg of ATG was given I.V. daily over 7 days at which time the MLN were cultured. To assess the degree of T cell depletion induced by ATG, sections of the spleen, MLN, and small intestine of control animals and those given 3 mg ATG/day (N = 3) for 7 days were examined by immunofluorescent microscopy using fluorescein-labeled monoclonal antibodies to the cell surface antigens Thy 1, Lyt 1, L3T4, and Lyt 2 (Figs. 1 and 2). These studies demonstrated that this dose of ATG completely depleted these T cell phenotypes from the spleen and small intestine. Identical results were obtained from spleens stained with anti-L3T4 and anti-Lyt 2 and for MLN stained with anti-Thy 1, anti-Lyt 1, anti-L3T4, and anti-Lyt 2. To ensure that the administered ATG was not simply binding *in vivo* to the cell surface antigen and therefore blocking the binding of the fluorescein tagged monoclonal antibodies, sections of spleen from control animals were exposed to a 1:10,000 dilution of ATG and then stained with a fluorescein-labeled monoclonal antihorse-Ig antibody. Sections of spleen from ATG treated animals were stained only with the antihorse-Ig antibody. Sections from control animals demonstrated intense fluorescence whereas those from ATG-treated animals had no fluorescence (not shown), demonstrating that actual depletion of T cells did occur. However, despite such a profound depletion of T cells from both the systemic T cell pool and the GALT, a significantly increased incidence of translocation was not induced at any of the doses of ATG used (Table 1).

TABLE 4. *Effect of ATG on the Incidence of* E. coli *C25 Translocation to Various Organs in Mice Monoassociated with* E. coli *C25**

Treatment	No. of Organs with Translocation of *E. coli* C25/Total No. of Mice			
	MLN	Spleen	Liver	Peritoneal Cavity
Monoassociation alone (control)	8/8	1/8	0/8	1/8
Monoassociation + ATG	8/8	0/8	0/8	ND

* BALB/c mice with a conventional cecal microflora were gut decontaminated for 4 days with oral bacitracin and streptomycin sulfate and were then orally monoassociated with *E. coli* C25 for 2 days; 3 mg/d of ATG was given over this 6-day period, and the organs were cultured on day 7.

Cecal flora studies showed no effect of CSA, anti-L3T4, or ATG on the number of gram-positive, gram-negative, or anaerobic bacteria per gram of cecum (data not shown).

Translocation of Indigenous Gastrointestinal Bacteria in Nude Mice

To assess whether T cell-deficient nude mice have an intrinsic defect in the ability of their GI tract to prevent translocation, the MLN, spleen, liver, and peritoneum were cultured in heterozygous (nu/+) and nude (nu/nu) littermates without prior intestinal flora manipulations. Table 2 shows that translocation of indigenous gut bacteria to the MLN, liver, spleen, or peritoneum in either heterozygous or nude mice was not occurring. However, an unexpected finding was that the cecal flora differed quantitatively from that found in Swiss Webster mice of comparable age (Table 3). The normal level of facultative gram-negative bacteria was significantly lower in the heterozygous and nude mice compared to the higher level observed in the Swiss Webster mice. Total anaerobic and gram-positive bacteria, however, were similar. This may partially account for the lack of translocation of gram-negative bacteria in the immunodeficient nude mice.

TABLE 3. *Average Log_{10} ± SE Viable Bacteria per Gram of Cecum in Conventionally Reared Swiss Webster Mice, in Balb/c T Cell-deficient (Nude) mice, and Their Heterozygous (Thymic) Littermates*

Type of Mouse	Strict Anaerobes	Aerobic and Facultative Gram-positive Bacteria	Aerobic and Facultative Gram-negative Bacilli	Average Cecal Weight (g)
Heterozygote (N = 4)	10.3 ± 0.3	7.4 ± 0.7	3.7 ± 1.0	0.72 ± 0.07
Nude (N = 4)	9.7 ± 0.5	7.4 ± 0.4	3.7 ± 1.0	0.56 ± 0.10
Swiss Webster (N = 4)	9.2 ± 1.4	6.9 ± 0.6	6.7 ± 1.7	0.50 ± 0.20

TABLE 5. *Effect of ATG on the Cecal Bacterial Counts of Mice Monoassociated with* E. coli *C25**

Treatment	Average Log_{10} ± SE Cecal Bacteria per Gram (N = 4)			Average Cecal Weight (g)
	E. coli C25	Strict Anaerobes	Aerobic and Facultative Gram-positive	
Monoassociation alone (control)	9.7 ± 0.3	0	1.0 ± 0.1	1.0 ± 0.1
Monoassociation + ATG	9.8 ± 0.1	0	1.03 ± 0.1	1.0 ± 0.1

* BALB/c mice with a conventional cecal microflora were gut decontaminated for 4 days with oral bacitracin and streptomycin sulfate and were then orally monoassociated with *E. coli* C25 for 2 days; 3 mg/d of ATG was given over this 6-day period, and the organs were cultured on day 7.

Effect of T Cell Deficiency on the Translocation of E. coli *C25*

Since neither T cell inhibition (CSA) or depletion (anti-L3T4, ATG, nude mice) induced translocation in mice with a normal intestinal flora, the influence of T cell immunity on translocation was next examined in mice monoassociated with *E. coli* C25. By decontaminating the GI tract with oral bacitracin and streptomycin for 4 days and then adding streptomycin-resistant *E. coli* C25 to the drinking water for 2 days, intestinal overgrowth of *E. coli* C25 to abnormally high levels occurs. Such intestinal overgrowth (monoassociation) leads to translocation of *E. coli* C25 to the MLN in 80–100% of animals but not to other organs.[8] Therefore, in animals with intact host defenses, the MLN is the point at which translocation is contained. To test the hypothesis that depression of T cell immunity would impair the ability of the MLN to contain translocating bacteria, immunocompetent BALB/c mice were given 3 mg/d of ATG during the 6-day period of monoassociation with *E. coli* C25 (total of six doses). Control mice received saline injections. On day 7 the MLN, spleen, liver, and peritoneum were cultured and the incidence of translocation of *E. coli* C25 to each organ was determined. Immunofluorescent antibody studies of the spleen, MLN, and intestine (N = 3 for control and ATG-treated mice) were also repeated and showed the same degree of depletion of Thy 1, Lyt 1, L3T4, and Lyt 2 cells as shown in Figures 1 and 2.

Table 4 shows that treatment with ATG had no influence on the incidence of translocation of *E. coli* C25 to the MLN when compared with control animals. In addition, complete depletion of the systemic and GALT T cell pool did not promote spread of *E. coli* C25 beyond the MLN to the spleen, liver, or peritoneum. Since the cecal levels of *E. coli* C25 were the same in both control and ATG-treated animals (Table 5), both groups had an equal susceptibility to translocate.

To further evaluate the ability of the suppression of T cell function to promote spread of translocating bacteria, we next studied BALB/c nude mice with a normal gut flora (conventional) and germ-free BALB/c nude mice. Conventional BALB/c nude mice and their heterozygous littermates were monoassociated with *E. coli* C25 as above. Cultures of the MLN, spleen, liver, and peritoneum on day 7 again showed the expected translocation of *E. coli* C25 to the MLN, but again, no spread of *E. coli* C25 to the spleen, liver, or peritoneum was observed (Tables 6 and 7).

Germ-free mice, because they are isolated from the external environment from birth, have a sterile GI tract. Therefore, oral inoculation with *E. coli* C25 without prior antibiotic treatment leads to intestinal overgrowth and translocation to the MLN in 90–100% of the animals. Although translocation to the MLN reliably occurs in immunocompetent germ-free mice, spread of *E. coli* to the spleen, liver, or peritoneum does not.[9] To assess whether GALT T cell immunity is important in the generation of a protective immune barrier to the systemic escape of bacteria, germ-free nude mice and their heterozygous littermates were orally monoassociated with *E. coli* C25 for 2 days and on the third day the MLN, spleen, and liver were cultured. Despite the presence of extremely high levels of *E. coli* C25 in the ceca of both the heterozygous and nude mice, translocation was again confined to the MLN alone (Tables 8 and 9).

Although spread of *E. coli* C25 beyond the MLN did not occur in either the ATG-treated, conventional nude,

TABLE 6. *Effect of Monoassociation with* E. coli *C25 in T Cell-deficient BALB/c Nude Mice**

Type of Mouse	No. of Organs with Translocation of *E. coli* C25/Total no. of Mice			
	MLN	Spleen	Liver	Peritoneal Cavity
Heterozygote	4/7	1/7	0/6	0/7
Nude	7/7	0/7	0/7	0/7

* BALB/c T cell deficient nude mice and their heterozygous littermates were gut decontaminated for 4 days with oral bacitracin and streptomycin sulfate and were then orally monoassociated with *E. coli* C25 for 2 days. On day 7 the organs were cultured.

TABLE 7. *Cecal Bacterial Counts in T Cell-deficient Nude Mice and Their Heterozygous Littermates after Monoassociation with* E. coli *C25*

Type of Mouse	Average Log_{10} ± SE Cecal Bacteria per Gram			Average Cecal Weight (g)
	E. coli C25	Strict Anaerobes	Aerobic and Faculatative Gram-positives	
Heterozygotes (N = 4)	10.8 ± 0.4	1.9 ± 2.1	4.5 ± 0.5	0.88 ± 0.02
Nude (N = 3)	10.6 ± 0.3	1.4 ± 2.4	4.7 ± 0.3	0.69 ± 0.14

or germ-free nude mice, a consistent finding in all three experiments was that the average number of *E. coli* C25 per MLN was higher than that in control animals (Table 10).

Effect of Treatment with Interleukin-2 on Translocation of E. coli *C25*

To examine the influence of augmented cellular immune function on bacterial translocation, BALB/c mice were monoassociated with *E. coli* C25 and during the 2 days of oral exposure to *E. coli* C25, IL-2 was given intraperitoneally either every 8 hours (25,000 units per dose, total of six doses) or as a single bolus dose of 150,000 units on either the first or second day of exposure to *E. coli* C25. Cultures of the MLN showed that IL-2 significantly decreased the overall incidence of translocation of *E. coli* C25 to the MLN compared to control animals only when given every 8 hours. Giving IL-2 as a bolus of 150,000 had no effect on either the incidence or number of *E. coli* C25 per MLN (Table 11).

Discussion

Despite the continuous exposure of its epithelial surface to the hostile microbial environment of its lumen, the GI tract maintains a highly effective barrier to the systemic entrance of gut microbes. Since the composition of the normal cecal and ileal flora consists of approximately 10^{10} anaerobic bacteria/g, 10^{5-7} aerobic and facultative gram-negative bacteria/g, and 10^{6-8} aerobic and facultative gram-positive bacteria/g, this barrier must be relatively nonselective and indifferent to the extreme degree of microbial and antigenic diversity. On the other hand, it must also be capable of selectively responding to microbial pathogens that use the gut as their portal of entry.

The GI tract's defense against translocation appears to involve two components. The first is a nonspecific mechanical and chemical barrier. This is composed of the lining mucosal cells with their tight intercellular junctional complexes, the mucus layer, which overlays the epithelium, and, finally, the indigenous gut bacteria, particularly the anaerobic component. The large population of anaerobes normally present appears to play a particularly important role in the normal control of other resident gut bacteria.[10] Due to their high numbers, they prevent overgrowth of other gram-negative and gram-positive bacteria within the intestine and, by secreting large amounts of short chain fatty acids within the mucus layer overlying the epithelium, they inhibit the adherence of other potential pathogens, such as *C. albicans*.[11]

The second component of the GI tract's defense system is the immunologic defense or gut-associated lymphoid tissue (GALT). The GALT is composed of: (1) a diffuse population of T-helper cells, B cells, and plasma cells located throughout the lamina propria of the intestine, and (2) Peyer's patches (in the small intestine) and lymphoid nodules (in the small and large intestine). Although Peyer's patches and lymphoid nodules differ in size, both are histologically and functionally similar.[12] Their luminal surface is lined by a specialized phagocytic cell referred to as an M (for microfold) cell.[13] Below the M cell is a population of macrophages and below these are lymphoid follicles that are composed of T and B cells (Fig. 3).

TABLE 8. *Effect of Monoassociation on Translocation of* E. coli *C25 in Germ-free T Cell-deficient BALB/c Nude mice and Their Heterozygous Littermates**

Type of Mouse	No. of Organs with Translocation of *E. coli* C25/Total No. of Mice		
	MLN	Spleen	Liver
Germ-free heterozygote	9/10	0/10	0/10
Germ-free nude	8/10	0/10	1/10

* Mice were orally monoassociated with *E. coli* C25 for 2 days, and on day 3 the organs were cultured.

TABLE 9. *Cecal Counts ± SE of* E. coli *C25 in Germ-free T Cell-deficient Nude Mice and Their Heterozygous Littermates after Oral Monoassociation with* E. coli *C25 for 2 Days*

Type of Mouse	Average Log_{10} *E. coli* C25 per Gram of Cecum	Average Cecal Weight (g)
Germ-free heterozygotes (N = 4)	12.2 ± 0.2	0.78 ± 0.04
Germ-free nude (N = 4)	12.2 ± 0.2	0.59 ± 0.03

By virtue of their unique structure, Peyer's patches and lymphoid nodules serve as the focal point for the induction of immune responses to foreign antigens. After uptake of a foreign antigen by an M cell, it is transferred without alteration to the underlying macrophages. The macrophages are thought to process the antigen, present it to the lymphocytes of the surrounding lymphoid nodules, following which antigen-specific T and B lymphoblasts are generated. These cells leave the intestine, pass through the systemic circulation, and then *home* to the lamina propria of the intestine where they mature to T-helper/inducer cells, B cells, and plasma cells, which are committed to the secretion of antigen-specific IgA into the intestine (Fig. 4).[12] Despite the presence of such an elaborate system for the generation of luminal IgA, the presence of IgA does not appear to be essential for the maintenance of an immunologic defense against translocation of indigenous gut bacteria because patients with selective IgA deficiency do not have an increased incidence of infections caused by the bacteria normally found in the gut.[14] However, IgA is extremely important in the defense against pathogens, which have the gut as their target organ (*e.g., Shigella* sp).[15]

Despite the presence of the both the mechanical/chemical barrier and the GALT, systemic translocation of indigenous gut bacteria does occur under a variety of circumstances. Escape of bacteria to the liver via the portal venous system appears to occur only when there has been an extreme insult (such as intestinal ischemia) to the integrity of the intestine.[16] However, translocation of bacteria from the gut lumen to the draining mesenteric lymph nodes can occur without direct damage to epithelial integrity. Van der Waaij et al. first noted in 1972 that the administration of oral antibiotics to mice would lead to translocation of bacteria to the MLN.[17] Since then it has been shown that disruption of the normal gut flora by the administration of selective antibiotics allows overgrowth of the remaining bacteria within the intestine and that such bacterial overgrowth often leads to translocation to the MLN.[18]

Translocation of indigenous gut bacteria to the MLN also occurs after a variety of other insults, such as following the administration of lipopolysaccharide,[19] after large body surface area burns,[20] with physical disruption of the gut mucosal barrier,[21] and after the administration of prednisone and cyclophosphamide.[22] Many of these situations have as part of their pathophysiology alterations in intestinal blood flow (LPS, burns with loss of fluid) or cellular function (cyclophosphamide), both of which can damage the integrity of the GI tract. Although the maintenance of an intact epithelial layer is critical in preventing translocation, the role of the GALT, in particular the role of T cell immunity, in the defense against translocating bacteria is unknown.

TABLE 10. *Effect of T Cell Deficiency on the No. of Translocating* E. coli *C25 Within the MLNs of Mice Monoassociated with* E. coli *C25*

Group Monoassociated with *E. Coli* C25	Average No. (±SE) *E. Coli* C25 per MLN	P Value*
ATG-treated		
Saline (N = 8)	21 ± 4	0.02
ATG (N = 8)	68 ± 16	
Conventional nude		
Heterozygotes (N = 7)	11 ± 6	0.07
Nude (N = 10)	40 ± 20	
Germ-free nude mice		
Heterozygotes (N = 10)	36 ± 8	0.15
Nudes (N = 10)	65 ± 17	

* Wilcoxon rank sum test.

In these studies we first sought to determine whether T cell dysfunction would induce translocation of indigenous gut bacteria in mice with a conventional cecal flora. Neither T-helper cell inhibition (CSA) or depletion (anti-L3T4 antibody), nor depletion of other T cells such as Lyt 2+ cells (T cytotoxic/suppressor cells) with ATG induced translocation. Because these alterations of T cell function were on a short-term basis (over a maximum of 14 days), the incidence of translocation to the

TABLE 11. *Effect of IL-2 on the Translocation of* E. coli *C25 to the MLN**

IL-2 Protocol	No. of Mice with Translocation of *E. coli* C25 to the MLN/ Total (%)	P Value†
Group I: 25,000 units every 8 hours		
HBSS	22/26 (85)	0.02
IL-2	14/27 (52)	
Group II: 150,000 units on day 1		
HBSS	10/10 (100)	NS
IL-2	10/10 (100)	
Group III: 150,000 units on day 2		
HBSS	8/9 (89)	NS
IL-2	9/10 (90)	

* BALB/c mice with a conventional cecal microflora were gut decontaminated for 4 days with oral bacitracin and streptomycin sulfate and were then orally monoassociated with *E. coli* C25 for 2 days. During the 2 days of oral *E. coli* C25 monoassociation mice received 150,000 units of IL-2 as: 25,000 units every 8 hours (Group I), a bolus dose on day one of *E. coli* C25 monoassociation (Group II); and a bolus dose on day 2 of *E. coli* C25 monoassociation (Group III).

† HBSS *versus* IL-2-treated mice by chi square analysis with a Yates continuity correction.

HBSS-Hanks balanced salt solution.

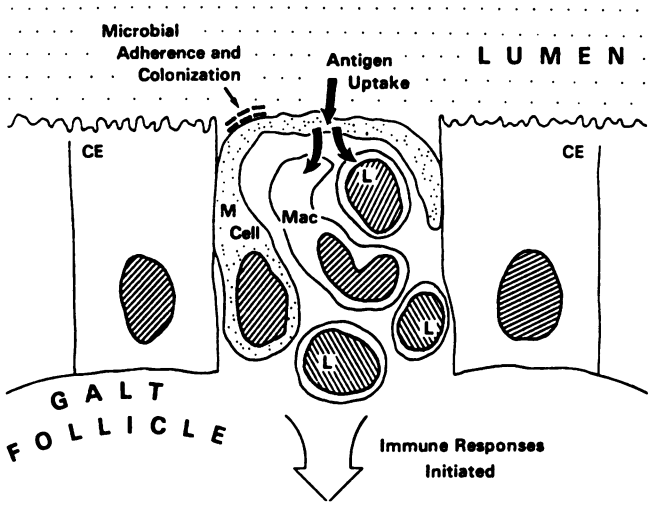

FIG. 3. Peyer's patch in the intestine. An M cell, the surface of which is in contact with the contents of the intestinal lumen, is surrounded by subepithelial macrophages (Mac) and by lymphocytes (L). M cells are important sites of antigen and microbe uptake by the GALT.

MLN, liver, and spleen in T cell-deficient nude mice was determined and was also found to be essentially zero. This, combined with the experiments utilizing CSA, anti-L3T4 antibody, and ATG, clearly demonstrates that suppression of T cell function or number has little effect on the ability of the GI tract to prevent translocation in animals in which gut microflora is unaltered and in which there is no other predisposition to

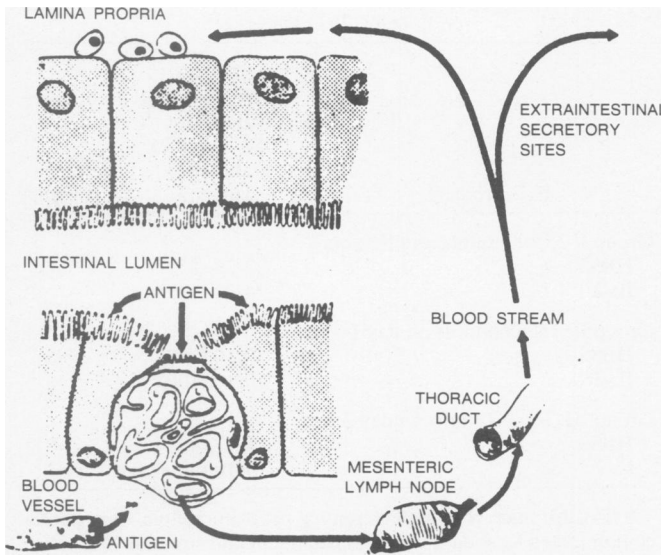

FIG. 4. Schematic representation of the induction of an immune response within the GALT. After antigen is processed by a Peyer's patch, antigen-specific T and B lymphoblasts enter the systemic circulation via the thoracic duct and then home to the lamina propria of the intestine. There they mature to antigen-specific T cells, B cells, and plasma cells, which are committed to the production of IgA for secretion into the intestinal lumen.

translocate. It would appear then that in animals without other predisposing factors to translocate (such as bacterial overgrowth or disruption of epithelial integrity), T cell immunity is not of primary importance in the prevention of bacterial translocation.

Under most of the experimental circumstances in which translocation occurs, it has been found that bacteria translocate most often to the MLN.[9] Only with severe insults to the intestine (ischemia or a combination of bacterial overgrowth and 20% body surface area burns)[20] will bacteria seed systemic sites such as the liver and spleen. Since, in the absence of direct intestinal damage, bacterial translocation is limited to the MLN, it appears that local factors within the MLN (for example T cell lymphokines) may exert a significant influence on the containment of bacteria that are translocating to the MLN.

To examine this question, a model of translocation was used which reliably induces translocation (in immunocompetent mice) of bacteria only to the MLN and rarely (<10%) to the liver or spleen. Monoassociation of (1) immunocompetent mice treated with 3 mg ATG/d for 6 days, (2) T cell-deficient nude mice with a conventional gut flora prior to monoassociation with *E. coli* C25, and (3) T cell-deficient nude germ-free mice with *E. coli* C25 yielded an equivalent incidence of translocation to the MLN. However, in all three experiments the MLN continued to be the site at which translocating *E. coli* C25 was contained since the spleen, liver, and peritoneum were invariably sterile. These results demonstrate that the MLN does, with this degree of translocation, serve as a point where translocating bacteria are entrapped and that T cells or their secreted factors play a small role in the prevention of systemic bacterial spread.

A rather striking and consistent trend noted in these experiments, however, was that suppression of T cell function did increase the average number of viable *E. coli* C25 per MLN. Although statistical significance was not achieved in the experiment in which germ-free nude mice were monoassociated, the trend was similar. The mechanism of this increase in bacteria per MLN is unknown. Whether due to an increased rate of translocation from the intestine or due to an impairment of the microbicidal environment of the lymph node, the results suggest that defects in T cell immunity may make the MLN a focus of persistent infection when there is translocation to it from whatever cause.

Finally, the influence of T cell stimulation on the translocation of *E. coli* C25 was investigated through the systemic administration of recombinant IL-2. IL-2 is a lymphokine produced by T-helper cells that stimulates the growth of activated lymphocytes *in vitro*. When administered *in vivo* IL-2 has a variety of effects including

enhancement of T helper and T cytotoxic cell reactivity to antigens,[23] reversal of several T cell immunodeficiencies in nude mice,[24,25] and the generation of cells that have the ability to lyse tumors *in vitro*.[26]

We found that a total dose of 150,000 units significantly decreased the overall incidence of translocation of *E. coli* C25 to the MLN. To be effective, it was necessary to administer the 150,000 units in divided doses of 25,000 units every 8 hours during the 2-day period of oral monoassociation with *E. coli* C25. A single bolus dose of 150,000 units either on the first or second day of *E. coli* C25 monoassociation was ineffective at reducing translocation. Since during the 2 days of monoassociation with *E. coli* C25 there presumably is continuous translocation of *E. coli* C25 to the MLN, and since the serum activity of an intraperitoneal dose of IL-2 persists for approximately 2–3 hours,[27] it is not surprising that the single bolus dose of 150,000 units was ineffective in decreasing translocation. However, since the immediate effect of the single bolus dose of 150,000 units on translocation was not tested by culturing the MLN within hours after IL-2 administration, it is possible that a decrease in the translocation of *E. coli* C25 may have been missed.

What role the *in vivo* generation of LAK cells played in diminishing translocation of *E. coli* C25 to the MLN is unknown. Although the cellular origin of LAK cells is at present unresolved, it appears that a substantial number are formed from preexisting natural killer (NK) cells.[28] To date, no studies of any possible antimicrobial activity of LAK cells have been performed, but a recent report has shown that a subpopulation of NK cells does possess phagocytic and microbicidal capabilities for the gram-positive microbe *Staphylococcus aureus*.[29] However, these same NK cells were unable to phagocytose either *E. coli* or *Pseudomonas aeruginosa*. Whether treatment with IL-2 can either expand this population of NK cells or induce them to become phagocytic for gram-negative bacteria is speculative, but given the intense degree of proliferation of LAK cells within the MLN that occurs after systemic IL-2 administration,[26] this remains a possibility.

An alternative explanation is that IL-2 may have enhanced the specific immune response to *E. coli* C25 within the GALT. Given the brief 48-hour period of exposure to IL-2, it seems unlikely that an increased secretion of IgA specific for *E. coli* C25 into the gut lumen would have occurred. However, within the MLN antigen-specific lymphocytes already exposed to *E. coli* C25 may be more rapidly induced in the presence of IL-2, and, if combined with a general increase (induced by IL-2) in the release of macrophage activating lymphokines (such as gamma interferon), there could be an increased microbicidal capacity of macrophages within the lymph node leading to increased killing of *E. coli* C25. Relevant to this concept is the recent demonstration by Holter et al. that gamma interferon and lipopolysaccharide can induce IL-2 receptors on peripheral blood monocytes and that, if subsequently exposed to IL-2, the monocytes display increased H_2O_2 activity, suggesting an increase in their microbicidal activity.[30] Regardless of the specific mechanism, it is likely that the phenomenon of decreased translocation of *E. coli* C25 after treatment with IL-2 is due to a local process within the MLN and not within the intestine since *in vivo* administration of IL-2 has been shown to have little, if any, proliferative effect on lymphocytes within the intestine.[26]

In conclusion, T cell immunity appears to have little effect on the prevention of the movement of bacteria from the gut lumen to the MLN. However, by influencing either the antibody response to translocating bacteria or the local microbicidal activity of the MLN against translocating bacteria, normal T cell function does appear to have a modest impact on the ability of the GALT to effectively deal with translocating bacteria. In a critically ill surgical patient who is receiving systemic antibiotics (the majority of which have a marked impact on the composition of the intestinal flora), combined with ileus (with its attendant stasis of gut contents), malnutrition (with impaired ability of the gut epithelial cells to renew themselves), and shock or low flow states (with reduction in blood flow to the intestines), the function of the host's immune system, particularly T cell function within the lymph nodes draining the gut, may assume importance in the ability to control translocating gut bacteria.

References

1. Marshall JC, Christou NV, Horn RV, Meakins JL. The microbiology of multiple organ failure: the proximal GI tract as an occult reservoir of pathogens. Arch Surg, in press.
2. Lanser ME. Immunobiologic consequences of trauma and sepsis. *In* Siegel JM, ed. Trauma, Emergency Surgery and Critical Care. New York: Churchill Livingstone, 1987:389–409.
3. Christon NV. Host defense mechanisms in surgical patients: a correlative study of the delayed hypersensitivity skin-test response, granulocyte function and sepsis. Can J Surg 1985; 28:39–46.
4. Platt JL, LeBien TW, Michael AF. Interstitial mononuclear cell populations in renal graft rejection: identification by monoclonal antibodies in tissue sections. J Exp Med 1982; 155:17–30.
5. Shevach EM. The effects of cyclosporin A on the immune system. Annu Rev Immunol 1985; 3:397–423.
6. Dialynas DP, Wilde DB, Marrack P, et al. Characterization of the murine antigenic determinant, designated L3T4a, recognized by monoclonal antibody GK1.5: expression of L3T4a by functional T cell clones appears to correlate primarily with class II MHC antigen reactivity. Immunol Rev 1983; 74:29–55.
7. Wofsy D, Seaman WE. Analysis of the function of L3T4 + T cells

by in vivo treatment with monoclonal antibody to L3T4. Immunol Res 1986; 5:97–105.
8. Maejima K, Shimoda K, Berg RD. Assessment of mouse strain on bacterial translocation from the gastrointestinal tract. Exp Anim 1984; 33:345–349.
9. Steffen EK, Berg RD. Relationship between cecal population levels of indigenous bacteria and translocation to the mesenteric lymph nodes. Infect Immun 1983; 39:1252–1259.
10. Wells CL, Maddaus MA, Reynolds CM, et al. The role of the anaerobic flora in the translocation of aerobic and facultative anaerobic intestinal bacteria. Infect Immun, 1987; 55:2689–2694.
11. Kennedy MJ, Voly PA. Ecolory of *Candida albicans* gut colonization: inhibition of candida adhesion, colonization, and dissemination from the gastrointestinal tract by bacterial antagonism. Infect Immun 1985; 49:654–663.
12. Streilein JW, Stein-Streilein J, Head J. Regional specialization in antigen presentation. *In* Phillips SM, Escobar MR, eds. The Reticuloendothelial System: a Comprehensive Treatise. New York: Plenum, 1987:37–93.
13. Wolf JL, Rubin DH, Finberg R, et al. Intestinal M cells: a pathway for entry of reovirus into the host. Science 1981; 212:471–472.
14. Underdown BJ, Schiff JM. Immunoglobulin A: strategic defense initiative at the mucosal surface. Annu Rev Immunol 1986; 4:389–417.
15. Keren DF, Holt PS, Collins HH, et al. The role of Peyer's patches in the local immune response of rabbit ileum to live bacteria. J Immunol 1978; 120:1892–1896.
16. Bennion RS, Wilson SE, Williams RA. Early portal anaerobic bacteremia in mesenteric ischemia. Arch Surg 1984; 119:151–155.
17. Van der Waaij D, Berhuis-deVices JM, Lekkerkerk-van der Wees JEC. Colonization resistance of the digestive tract and the spread of bacteria to the lymphatic organs in mice. J Hyg Camb 1972; 70:335–342.
18. Berg RD. Promotion of the translocation of enteric bacteria from the gastro-intestinal tracts of mice by oral treatment with penicillin, clindamycin, or metronidazole. Infect Immun 1987; 33:854–861.
19. Deitch EA, Berg RD, Specian RO. Endotoxin promotes the translocation of bacteria from the gut. Arch Surg 1987; 122:185–190.
20. Maejima K, Deitch EA, Berg RD. Bacterial translocation from the gastrointestinal tract of rats receiving thermal injury. Infect Immun 1984; 43:6–10.
21. Morehouse JL, Specian RD, Stewart JJ, Berg RD. Translocation of indigenous bacteria from the gastrointestinal tract of mice after oral ricinoleic acid treatment. Gastroenterology 1986; 91:673–682.
22. Berg RD. Bacterial translocation from the gastrointestinal tract of mice receiving immunosuppressive chemotherapeutic agents. Am Microbiol 1983; 8:285–292.
23. Rosenberg SA, Spiess PJ, Schwarz S. In vivo administration of interleukin-2 enhances specific alloimmune responses. Transplantation 1983; 35:631.
24. Stotter H, Rude E, Wagner H. T cell factor (interleukin-2) allows in vivo induction of T helper cells against heterologous erythrocytes in athymic (nu/nu) mice. Eur J Immunol 1980; 10:719.
25. Wagner H, Hardt C, Heeg K, et al. T-cell derived helper factor allows in vivo induction of cytotoxic T cells in nu/nu mice. Nature 1980; 284:278.
26. Ettinghausen SE, Lipford EM, Mule JJ, Rosenberg SA. Systemic administration of recombinant interleukin 2 stimulates in vivo lymphoid cell proliferation in tissues. J Immunol 1985; 135:1488–1497.
27. Donohue JH, Rosenberg SA. The fate of interleukin-2 after in vivo administration. J Immunol 1983; 130:2203–2208.
28. Mules JJ, Yang JC, Afreniere RL, et al. Identification of cellular mechanisms operational in vivo during the regression of established pulmonary metastases by the systemic administration of high-dose recombinant interleukin 2. J Immunol 1987; 139:285–294.
29. Abo T, Sugawara S, Amenomori A, et al. Selective phagocytosis of gram-positive bacteria and interleukin 1-like factor production by a subpopulation of large granular lymphocytes. J Immunol 1986; 136:3189–3197.
30. Holter W, Goldman CK, Casabo L, et al. Expression of functional IL-2 receptors by lipopolysaccharide and interferon-gamma stimulated human monocytes. J Immunol 1987; 138:2917–2922.